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Surveillance for High Pathogenicity Avian Influenza Virus in Wild Birds in the Pacific Flyway of the United States, 2006–2007

Robert J. Dusek,[AF] J. Bradley Bortner,[B] Thomas J. DeLiberto,[C] Jenny Hoskins,[B] J. Christian Franson,[A] Bradley D. Bales,[D] Dan Yparraguirre,[E] Seth R. Swafford,[C] and Hon S. Ip[A]

[A]U.S. Geological Survey, National Wildlife Health Center, Madison, WI 53711
[B]U.S. Fish and Wildlife Service, Portland, OR 97232
[C]U.S. Department of Agriculture/Animal and Plant Health Inspection Service, Wildlife Services, Fort Collins, CO 80521
[D]Oregon Department of Fish and Wildlife, Salem, OR 97303
[E]California Department of Fish and Game, Sacramento, CA 95814

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SUMMARY. In 2006 the U.S. Department of Agriculture, U.S. Department of Interior, and cooperating state fish and wildlife agencies began surveillance for high-pathogenicity avian influenza (HPAI) H5N1 virus in wild birds in the Pacific Flyway of the United States. This surveillance effort was highly integrated in California, Oregon, Washington, Idaho, Nevada, Arizona, Utah, and western Montana, with collection of samples coordinated with state agencies. Sampling focused on live wild birds, hunter-killed waterfowl during state hunting seasons, and wild bird mortality events. Of 20,888 samples collected, 18,139 were from order Anseriformes (waterfowl) and 2010 were from order Charadriiformes (shorebirds), representing the two groups of birds regarded to be the primary reservoirs of avian influenza viruses. Although 83 birds were positive by H5 real-time reverse transcription polymerase chain reaction (rRT-PCR), no HPAI H5N1 virus was found. Thirty-two virus isolates were obtained from the H5-positive samples, including low-pathogenicity H5 viruses identified as H5N2, H5N3, and H5N9.

RESUMEN. Muestreo para detectar influenza aviar de alta patogenicidad en aves silvestres de la ruta migratoria del Pacífico de los Estados Unidos, 2006–2007.

En el año 2006 el Departamento de Agricultura y el Departamento del Interior de los Estados Unidos, con la cooperación de agencias estatales de pesca y vida silvestre iniciaron un muestreo para detectar la influenza aviar de alta patogenicidad subtipo H5N1 en aves silvestres de la ruta migratoria del Pacífico de los Estados Unidos. Este proyecto estuvo integrado principalmente en California, Oregón, Washington, Idaho, Nevada, Arizona, Utah, y la parte oeste de Montana, con la recolección de muestras coordinada por las agencias estatales. El muestreo estuvo enfocado en aves silvestres vivas, aves acuáticas muertas por cazadores durante las estaciones de caza y en eventos de mortalidad de aves silvestres. De las 20,888 muestras recolectadas, 18,139 muestras fueron de aves del orden Anseriformes (aves acuáticas) y 2010 fueron de aves del orden Caradriformes (aves playeras), que representan los dos grupos de aves considerados como los principales reservorios de los virus de influenza aviar. Aunque 83 aves fueron positivas para la hemaglutinina 5 (H5) por la prueba de transcripción reversa y reacción en cadena de la polimerasa en tiempo real, no se encontró la presencia del virus de alta patogenicidad subtipo H5N1. Treinta y dos aislamientos fueron obtenidos de las muestras positivas a H5, incluyendo virus de baja patogenicidad identificados como H5N2, H5N3 y H5N9.

Key words: avian influenza, HPAI, H5N1, surveillance, United States

Abbreviations: AHY = after hatch year; HPAI = high-pathogenicity avian influenza; HY = hatch year; LPAI = low-pathogenicity avian influenza; NVSL = National Veterinary Services Laboratories; rRT-PCR = real-time reverse transcription polymerase chain reaction; USDA = United States Department of Agriculture

While wild aquatic birds are considered the principal agent for long-distance spread and maintenance of low-pathogenicity avian influenza (LPAI) viruses, they have rarely been involved in high-pathogenicity avian influenza (HPAI) outbreaks (1). Historically, only one HPAI outbreak occurred primarily in wild birds not associated with an HPAI outbreak in poultry (1,4). Considerable debate exists on the role wild birds have played in the current global spread of HPAI H5N1 (15,16,31,35). Most wild bird mortality due to HPAI H5N1 has been associated with mortality in domestic birds in urban or agricultural areas (14,24). However, experimental studies have shown that some wild birds can be infected with HPAI H5N1 without developing clinical signs and may be capable of transporting this virus (5,20).

A variety of mechanisms likely play a role in the geographic spread of HPAI H5N1 (1). However, since an outbreak of HPAI H5N1 that occurred primarily in bar-headed geese (*Anser indicus*) at Qinghai Lake in Western China in 2005, there is increasing evidence being compiled to support the hypothesis that wild birds are involved in the spread of HPAI H5N1 (10,26,27,34). Furthermore, recent phylogenetic analyses of HPAI H5N1 virus isolates from outbreaks in domestic poultry and migratory birds from other locations have demonstrated a close relationship with isolates from the Qinghai Lake outbreak (25,35,42).

While HPAI H5N1 virus strains currently circulating in Asia and other parts of the world have not yet been detected in the Western Hemisphere, evidence of gene flow between Europe/Asia and North America of LPAI viruses supports the possibility of its introduction via migratory birds (21,28). Owing to the potential impact of this virus on the U.S. poultry industry as well as the potential that genetic changes in the virus could trigger a pandemic, the U.S. government developed and instituted in 2006 the U.S. Interagency Strategic Plan (U.S. Strategic Plan) for the early detection of HPAI H5N1 (43). The goal of the strategic plan is to "describe the essential components of a unified national system for the early detection of HPAI, specifically highly pathogenic H5N1 avian

[F]Corresponding author. E-mail: rdusek@usgs.gov

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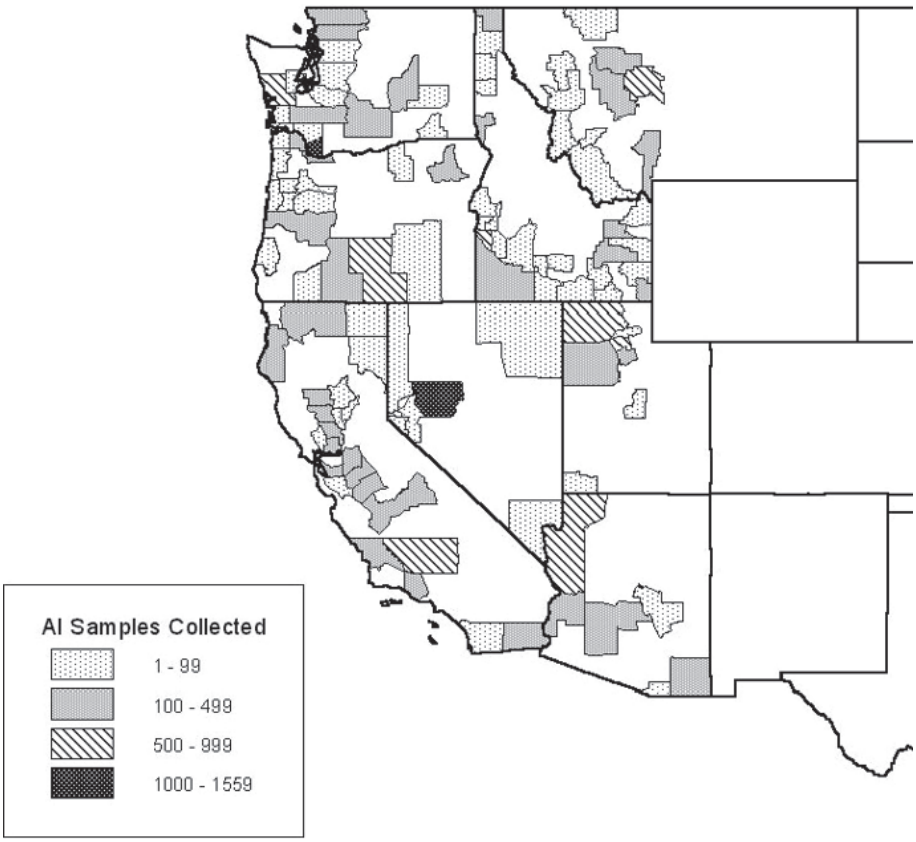

Fig. 1. Sampling locations for live bird and hunter-killed surveillance for high-pathogenicity avian influenza H5N1 in the Pacific Flyway, United States, April 2006–March 2007. (Base map from ESRI (2006), ArcMAP v. 9.2)

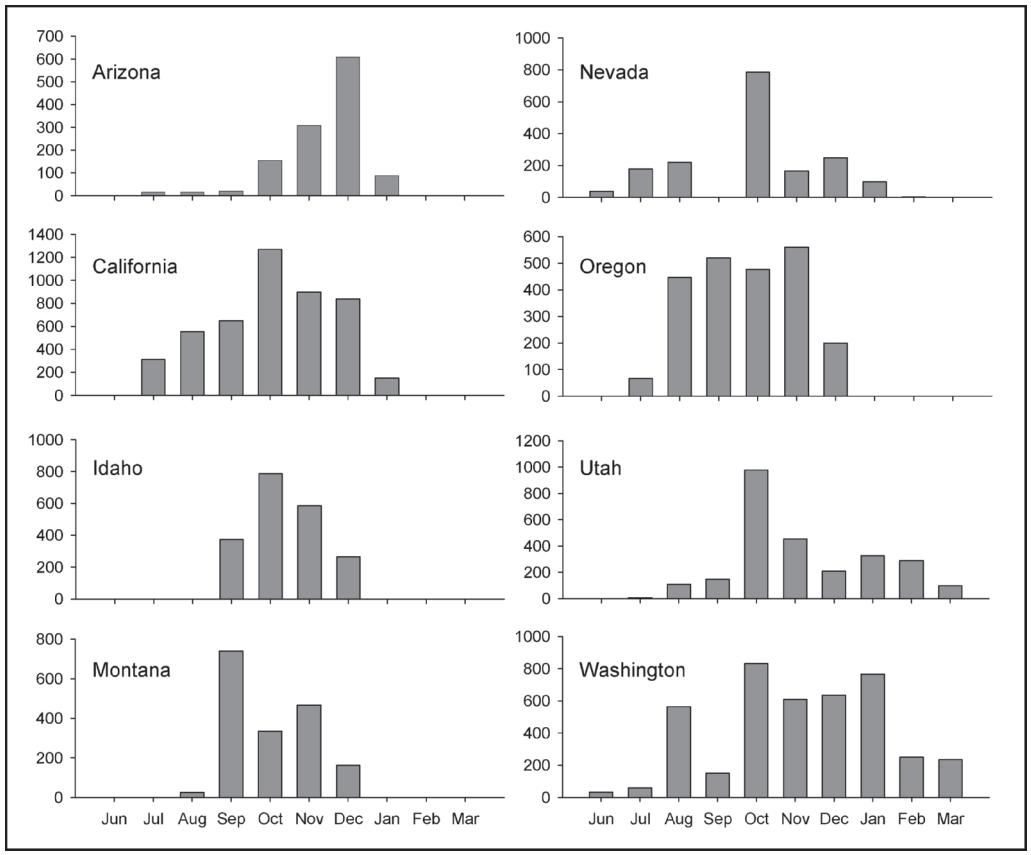

Fig. 2. Number of live bird and hunter-killed samples collected for high-pathogenicity avian influenza H5N1 surveillance in the Pacific Flyway, United States, by state, April 2006–March 2007.

Table 1. Live and hunter-killed bird species sampled during surveillance for high-pathogenicity avian influenza H5N1 in the Pacific Flyway, United States, April 2006–March 2007.

Family	Common name	Scientific name	Number sampled
Anatidae	Fulvous whistling-duck	*Dendrocygna bicolor*	1
	Trumpeter swan	*Cygnus buccinator*	7
	Tundra swan	*Cygnus columbianus*	334
	Greater white-fronted goose	*Anser albifrons*	224
	Snow goose	*Chen caerulescens*	393
	Ross goose	*Chen rossii*	2
	Black brant	*Branta bernicla*	391
	Cackling goose	*Branta hutchinsii*	717
	Aleutian cackling goose	*Branta hutchinsii leucopareia*	413
	Canada goose	*Branta canadensis*	289
	Wood duck	*Aix sponsa*	21
	Eurasian wigeon	*Anas penelope*	1
	American wigeon	*Anas americana*	1166
	Gadwall	*Anas strepera*	613
	Green-winged teal	*Anas crecca*	1880
	Mallard	*Anas platyrhynchos*	5978
	Northern pintail	*Anas acuta*	3844
	Blue-winged teal	*Anas discors*	47
	Cinnamon teal	*Anas cyanoptera*	39
	Northern shoveler	*Anas clypeata*	1330
	Unidentified teal	*Anas* sp.	2
	Canvasback	*Aythya valisineria*	20
	Redhead	*Aythya americana*	128
	Ring-necked duck	*Aythya collaris*	106
	Greater scaup	*Aythya marila*	15
	Lesser scaup	*Aythya affinis*	22
	Harlequin duck	*Histrionicus histrionicus*	1
	Surf scoter	*Melanitta perspicillata*	64
	White-winged scoter	*Melanitta fusca*	14
	Common goldeneye	*Bucephala clangula*	35
	Bufflehead	*Bucephala albeola*	23
	Common merganser	*Mergus merganser*	4
	Ruddy duck	*Oxyura jamaicensis*	15
Waterfowl total			18,139
Recurvirostridae	American avocet	*Recurvirostra americana*	1
Charadriidae	Semipalmated plover	*Charadrius semipalmatus*	39
	Killdeer	*Charadrius vociferus*	1
Scolopacidae	Short-billed dowitcher	*Limnodromus griseus*	37
	Long-billed dowitcher	*Limnodromus scolopaceus*	532
	Marbled godwit	*Limosa fedoa*	1
	Lesser yellowlegs	*Tringa flavipes*	1
	Ruddy turnstone	*Arenaria interpres*	1
	Red knot	*Calidris canutus*	2
	Sanderling	*Calidris alba*	12
	Western sandpiper	*Calidris mauri*	689
	Least sandpiper	*Calidris minuta*	125
	Dunlin	*Calidris alpina*	521
	Red-necked phalarope	*Phalaropus lobatus*	48
Shorebirds total			2010
Podicipedidae	Pied-billed grebe	*Podilymbus podiceps*	2
	Western grebe	*Aechmophorus occidentalis*	1
Ardeidae	Great blue heron	*Ardea herodias*	1
	Great egret	*Ardea alba*	2
Accipitridae	Swainson hawk	*Buteo swainsoni*	1
	Red-tailed hawk	*Buteo jamaicensis*	1
Tetraonidae	Spruce grouse	*Falcipennis canadensis*	1
Phasianidae	Ring-necked pheasant	*Phasianus colchicus*	5
Gruidae	Sandhill crane	*Grus canadensis*	162
Rallidae	American coot	*Fulica americana*	43
Corvidae	Common raven	*Corvus corax*	3
Other species total			222
Grand total			20,371

Table 2. Summary of H and N combinations detected from individual H5 rRT-PCR positive samples, Pacific Flyway, United States, April 2006–March 2007.

Species	H4N2	H4N3	H4N6	H5N2	H5N3	H5N9	H6N2	NVI[A]
Mallard (*A. platyrhynchos*)	2	1	2	16			1	34
Northern pintail (*A. acuta*)				1	2	1		4
Northern shoveler (*A. clypeata*)				2	1			6
American wigeon (*A. americana*)				2				3
Green-winged teal (*A. crecca*)								2
Snow goose (*C. caerulescens*)				1				0
Cackling goose (*B. hutchinsii*)								1
Tundra swan (*C. columbianus*)								1

[A]NVI = No virus isolated.

influenza, in migratory birds. While the immediate concern is a potential introduction of highly pathogenic H5N1 avian influenza into the U.S., the development of a system that is capable of detecting the introduction of all HPAI viruses through migratory birds would significantly improve the biosecurity of the Nation.'' As a part of that plan, surveillance for HPAI H5N1 in live and hunter-killed wild migratory birds was outlined, as well as investigation of its occurrence during morbidity and mortality events in wild birds. In addition, detection for LPAI H5 and H7 subtypes is included in the national surveillance efforts, since these viruses can be precursors to HPAI viruses in domestic poultry (30).

Alaska has been considered the most likely location for early introduction of HPAI H5N1 by migratory birds in North America (43). This is due to its proximity to Asia and the numbers of migratory birds that move between Asia and North America across the Bering Sea. The western continental United States (lower Pacific Flyway) is also considered an area of increased concern because of the numbers of species that breed in northern Russia and Alaska, in the same habitats as birds that migrate to Asia, and that migrate via the North American Pacific Flyway into or through the western United States. In 2006–2007 we began surveillance for HPAI H5N1 in wild birds in the lower Pacific Flyway of the United States. This report describes the development of the program and presents the results for the first surveillance season (April 1, 2006, through March 31, 2007).

MATERIALS AND METHODS

Planning. The 2006 U.S. Strategic Plan provided a national framework for surveillance for HPAI H5N1 in wild migratory birds throughout the United States (43). This plan recommended a flyway approach for conducting surveillance activities and prioritizing species for sampling. In the lower Pacific Flyway (Fig. 1), state wildlife agencies, the U.S. Fish and Wildlife Service, the U.S. Department of Agriculture—Wildlife Services, and partners refined the U.S. Strategic Plan into a Pacific Flyway Strategic Plan (Pacific Flyway Plan) for avian influenza surveillance sampling in 2006 (32). Prioritization of species targeted for sampling was based on expert knowledge of the ecology and spatial and temporal distribution of migratory birds throughout the flyway. Each of the eight states in the lower Pacific Flyway further stepped down the Pacific Flyway Plan into interagency state surveillance plans for implementing specific surveillance strategies.

Species selection, sample size, and temporal considerations. Wild bird species that use areas in both North America and Asia were identified in the U.S. Strategic Plan and were ranked based on five criteria (43). Briefly, ranking was done by assigning a score to each species based on each of the following factors: 1) the proportion of the population occurring in Asia, 2) contact with a known area affected by HPAI H5N1, 3) habitats used in Asia, 4) the population size in Alaska, 5) and the probability of obtaining a sufficient number of birds for sampling (43). Scores were summed to list species by priority. The Pacific Flyway Plan then identified *primary* species based on these criteria and that were available for sampling in the lower Pacific Flyway and *secondary* species that may comingle with these primary species. It was determined that monitoring of abundant secondary species, such as mallards (*Anas platyrhynchus*), would be useful if Asian HPAI H5N1 went undetected in primary species. Primary species sampled were tundra swan (*Cygnus columbianus*), lesser snow goose (*Chen caerulescens*), Pacific brant (*Branta bernicla*), Aleutian cackling goose (*Branta hutchinsii leucopareia*), northern pintail (*Anas acuta*), long-billed dowitcher (*Limnodromus scolopaceus*), red knot (*Calidris canutus*), ruddy turnstone (*Arenaria interpres*), western sandpiper (*Calidris mauri*), dunlin (*Calidris alpina*), and red-necked phalarope (*Phalaropus lobatus*). Secondary species sampled were cackling goose (*Branta hutchinsii*), greater white-fronted goose (*Anser albifrons*), mallard, American wigeon (*Anas americana*), green-winged teal (*Anas crecca*), and northern shoveler (*Anas clypeata*).

Sampling plans were developed for each state in the flyway to implement specific surveillance activities for the highest ranking species. Development of state-specific plans allowed experts to use local knowledge, such as targeting birds with known origins from Alaska (based on banding analyses or other information), to implement surveillance strategies efficiently at a fine scale. Local experts (primarily state and federal agency migratory bird biologists and managers) used their knowledge of spatial and temporal variation in migration timing, species abundance and availability, and habitat conditions to design sampling efforts to achieve target sample sizes. Surveillance in live birds and hunter-harvested birds often used existing field studies, management programs, and hunter check stations. The U.S. Strategic Plan did not dictate rigid sampling objectives but recommended general guidance of a target sample size of 200 birds from spatially or temporally segregated ''populations'' (e.g., northern pintails arriving in August and September in the San Joaquin Valley, California). This was derived under the assumption that the goal would be to detect a minimum prevalence of 1.5% with a 95% statistical power (43). In addition, mallards were targeted for sampling during the summer and early autumn because of their susceptibility to H5 and H7 AI subtypes (30,44). They were also abundant and easy to capture, so they could serve as sensitive indicators of HPAI H5N1 introduction (44,45). Other species were opportunistically sampled when they were captured in conjunction with planned sampling. Additional avian influenza sampling occurred during investigation of morbidity and mortality events, which occur annually in the Pacific Flyway.

Field sampling of wild birds. Capture locations for live sampled birds were selected based on known occurrence of high-priority species. Techniques for capturing birds varied but primarily involved rocket nets for waterfowl and larger shorebirds and mist nets for smaller shorebirds (6). Hunter-harvested birds were sampled during autumn and winter sport hunting seasons. Hunters were contacted at check stations at state or federal wildlife areas or boat ramps. Locations for sampling hunter-harvested birds were selected based on known harvest areas for high-priority species and existing harvest check stations.

Table 3. Results of virus isolation from H5 rRT-PCR positive samples collected during live bird and hunter-killed bird surveillance for high-pathogenicity avian influenza H5N1 in the Pacific Flyway, United States, April 2006–March 2007.

Common name	Scientific name	Sex	Age[A]	State	County	Date collected	V.I. subtype[B]	No. of birds
Tundra swan	*C. columbianus*	U	AHY	UT	Box Elder	Nov. 11, 2006	NVI[C]	1
Cackling goose	*B. hutchinsii*	M	HY	OR	Lane	Dec. 6, 2006	NVI	1
Snow goose	*C. caerulescens*	U	HY	MT	Teton	Nov. 4, 2006	H5N2	1
American wigeon	*A. americana*	F	HY	ID	Bingham	Oct. 7, 2006	NVI	1
		F	AHY	WA	Clark	Nov. 4, 2006	H5N2	1
		F	HY	OR	Columbia	Nov. 9, 2006	H5N2	1
		M	HY	OR	Coos	Nov. 18, 2006	NVI	1
		F	AHY	OR	Columbia	Nov. 21, 2006	NVI	1
Green-winged teal	*A. crecca*	M	HY	ID	Cassia	Oct. 7, 2006	NVI	1
		F	HY	UT	Davis	Nov. 11, 2006	NVI	1
Mallard	*A. platyrhynchos*	F	HY	WA	Grant	Jul. 22, 2006	NVI	1
		M	HY	NV	Churchill	Jul. 24, 2006	H5N2	5
		F	HY	NV	Churchill	Jul. 28, 2006	NVI	1
		M	HY	NV	Churchill	Jul. 28, 2006	H5N2	3
		M	HY	NV	Churchill	Jul. 28, 2006	NVI	1
		F	HY	WA	Yakima	Aug. 4, 2006	H4N3	1
		F	HY	WA	Yakima	Aug. 4, 2006	NVI	1
		F	U	WA	Yakima	Aug. 4, 2006	H4N6	1
		M	HY	WA	Yakima	Aug. 4, 2006	H4N6	1
		M	HY	WA	Yakima	Aug. 4, 2006	NVI	2
		F	HY	OR	Multnomah	Aug. 8, 2006	NVI	1
		F	HY	WA	Grant	Aug. 10, 2006	H5N2	1
		F	HY	WA	Grant	Aug. 18, 2006	NVI	1
		F	HY	WA	Yakima	Aug. 23, 2006	NVI	2
		F	HY	NV	Lyon	Aug. 24, 2006	H4N2	1
		M	HY	NV	Lyon	Aug. 24, 2006	H5N2	2
		M	HY	NV	Lyon	Aug. 24, 2006	NVI	1
		F	HY	OR	Multnomah	Aug. 29, 2006	NVI	1
		F	HY	WA	Skagit	Aug. 29, 2006	NVI	1
		M	HY	WA	Grant	Aug. 29, 2006	NVI	1
		M	HY	MT	Lake	Aug. 29, 2006	NVI	1
		M	HY	OR	Multnomah	Aug. 29, 2006	NVI	1
		F	HY	WA	Yakima	Aug. 30, 2006	NVI	2
		M	HY	WA	Grant	Aug. 30, 2006	NVI	1
		M	HY	WA	Yakima	Aug. 30, 2006	H5N2	1
		M	HY	WA	Yakima	Aug. 30, 2006	NVI	4
		M	AHY	AZ	Maricopa	Sep. 6, 2006	H5N2	1
		M	HY	ID	Jefferson	Sep. 6, 2006	NVI	1
		M	HY	ID	Kootenai	Sep. 7, 2006	H5N2	1
		M	HY	ID	Kootenai	Sep. 7, 2006	NVI	2
		M	HY	WA	Whatcom	Sep. 8, 2006	H5N2	1
		M	HY	MT	Cascade	Sep. 13, 2006	H6N2	1
		M	HY	MT	Cascade	Sep. 19, 2006	NVI	1
		M	HY	MT	Cascade	Sep. 20, 2006	H5N2	1
		F	AHY	UT	Davis	Oct. 7, 2006	NVI	1
		M	AHY	ID	Bonneville	Oct. 7, 2006	NVI	1
		M	U	UT	Box Elder	Oct. 7, 2006	NVI	2
		F	AHY	ID	Benewah	Oct. 9, 2006	H4N2	1
		M	AHY	ID	Jefferson	Oct. 11, 2006	NVI	1
		M	AHY	WA	Whatcom	Nov. 12, 2006	NVI	1
		M	AHY	MT	Gallatin	Nov. 30, 2006	NVI	1
Northern pintail	*A. acuta*	M	AHY	CA	Kern	Aug. 29, 2006	NVI	1
		F	AHY	MT	Cascade	Sep. 7, 2006	NVI	1
		F	HY	MT	Cascade	Sep. 14, 2006	H5N3	1
		M	HY	MT	Cascade	Sep. 14, 2006	H5N3	1
		M	HY	CA	Siskiyou	Oct. 3, 2006	H5N9	1
		F	HY	CA	Siskiyou	Oct. 5, 2006	H5N2	1
		M	AHY	AZ	Mohave	Oct. 28, 2006	NVI	1
		M	AHY	WA	Clark	Nov. 2, 2006	NVI	1
Northern shoveler	*A. clypeata*	F	AHY	UT	Davis	Oct. 7, 2006	H5N2	1
		F	HY	OR	Columbia	Oct. 24, 2006	NVI	1
		F	AHY	WA	Clark	Nov. 4, 2006	H5N2	1
		M	AHY	UT	Davis	Nov. 4, 2006	H5N3	1

Table 3. Continued.

Common name	Scientific name	Sex	Age[A]	State	County	Date collected	V.I. subtype[B]	No. of birds
		M	U	UT	Box Elder	Nov. 4, 2006	NVI	3
		F	AHY	WA	Clark	Nov. 7, 2006	NVI	1
		M	AHY	WA	Clark	Nov. 7, 2006	NVI	1

[A]AHY = after hatch year, HY = hatch year, U = unknown.
[B]V.I. subtype = virus isolate subtype.
[C]NVI = no virus isolated.

Virus samples were obtained by passing a sterile Dacron® or polyester swab around the interior of the cloaca to make contact with the mucosa. Each swab was immediately placed into an individually labeled vial containing 1.5 ml of either viral transport media (13) or brain heart infusion media (40). Samples were kept cool in the field and shipped to participating laboratories unfrozen by overnight courier on frozen ice packs within 48 h of collection or transferred to liquid nitrogen vapor shippers (−150 C) within 24 h and shipped on dry ice.

Carcasses collected during morbidity and mortality investigations were either shipped intact, either cooled or frozen, to a participating laboratory where cloacal swabs were collected or samples were collected in the field and stored and shipped as above.

Laboratory analyses. Molecular detection of avian influenza viruses was performed according to a uniform protocol by laboratories participating in the National Animal Health Laboratory Network (43). Samples were tested individually or pooled in groups of two to five (by species group and a sampling location and event) and tested within 72 hr of receipt. Briefly, viral RNA was extracted from a 50 µl aliquot of the cloacal swab media using the MagMax® Viral RNA extraction kit (Ambion, Austin, TX) according to manufacturer's instructions. Eight microliters of the recovered RNA was tested in the matrix gene real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the detection of avian influenza (40,43). Matrix gene rRT-PCR positive pools were subjected to additional testing in one of two ways. Either the pools were subjected to H5– and H7–specific rRT-PCR tests, or individual samples from the pools were tested using the matrix gene rRT-PCR test. In the latter case, individual samples testing matrix positive were then subjected to H5– and H7–specific rRT-PCR tests. All H5- and H7-positive individual samples or individual samples from H5- and H7-positive pools were sent to the U.S. Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in Ames, IA, for H5 or H7 confirmation, hemagglutinin and neuraminidase subtype determination using a panel of reference antisera, and pathogenicity determination. For virus isolation, all samples were tested individually and negative samples were passaged twice before they were declared negative. Pathogenicity was assessed using *in vivo* pathotyping or amino acid sequencing of the hemagglutinin proteolytic cleavage site according to the World Organization of Animal Health (Office Internationale des Epizooties) standards (2,36). In addition to these testing procedures, one laboratory further tested matrix rRT-PCR positive samples by an alternate H7 rRT-PCR (38,39), and positive samples were sent to USDA NVSL for further testing as stated above.

RESULTS

Samples were collected from eight western states: California, Oregon, Washington, Idaho, Nevada, Arizona, Utah, and western Montana (the portion that includes the counties of Hill, Chouteau, Cascade, Meagher, and Park, and all counties to the west; Fig. 1, Fig. 2). In total 20,371 wild bird samples were collected and tested during the 2006–2007 surveillance effort, representing 57 species, including 32 species of Anseriformes and 14 species of Charadriiformes (Table 1). Eleven additional species represented six bird orders and totaled 222 (1.1%) samples. Three species (mallard, northern pintail, and green-winged teal) accounted for 55.6% of the total sample. Of the total samples, 83 (0.41%) were positive by rRT-PCR analysis for the H5 subtype and represented eight species from the order Anseriformes, family Anatidae (Table 2, Table 3). Positive rRT-PCR H5 tests and virus isolations were obtained from all eight states participating in the surveillance effort. However, Nevada had the highest number of H5 virus subtypes identified with 10, all H5N2, primarily from a single county (Churchill) between 24 and 28 July. No H5-positive rRT-PCR sample tested positive for the N1 subtype. On further testing at NVSL, none of the samples tested positive for HPAI virus. A total of 32 rRT-PCR H5-positive samples yielded 5 virus isolates, with H5N2 being the most common subtype detected (Table 2). No samples tested positive for the H7 subtype by initial H7 rRT-PCR analysis. Testing of a subset of matrix rRT-PCR positive samples ($n = 359$) by an alternate H7 rRT-PCR method (38,39) identified 14 H7 rRT-PCR positive samples that resulted in 14 LPAI H7N3 virus isolations from four species (Table 4). Upon test of individual samples at NVSL that were initially from rRT-PCR H5-positive pools, 23 were virus positive but H5 rRT-PCR negative, including five false negative samples ($n = 250$, Table 5). Viral subtypes identified include H5N2 ($n = 3$) and H5N3 ($n = 2$), as well as H3N6 ($n = 1$), H3N8 ($n = 6$), H4N6 ($n = 10$), and H10N7 ($n = 1$).

In addition, samples from 115 morbidity or mortality events in the Pacific Flyway when 1 or more birds was found sick or dead were tested, resulting in the testing of 517 (mean = 4.5 per event, range 1–49) birds. A cluster of three events extended in duration from November 7, 2006, through March 27, 2007, in three counties in Washington State resulting in 101 samples from trumpeter swans (*Cygnus buccinator*, $n = 97$) and tundra swans ($n = 4$). No samples tested H5 or H7 rRT-PCR positive from morbidity or mortality events.

DISCUSSION

Our surveillance program was designed to target groups of bird species that are most often associated with avian influenza (orders Anseriformes and Charadriiformes) and therefore more likely to be found with HPAI H5N1 if it was present in wild North American birds. Sampling targeted geographic areas where these birds congregate during migration and in winter. This surveillance effort occurred in the Pacific Flyway region of the continental United States, where millions of water birds migrate through and winter with a portion of these birds originating from their breeding grounds or staging area in parts of Asia (northern Russia) and Alaska where they share habitats with birds that migrate along the East Asian and other Eastern Hemisphere flyways. More than 20,000 wild birds were sampled, representing live bird captures, hunter-killed birds, and wild bird mortality events. None of these samples tested positive for HPAI H5N1. All H5 rRT-PCR positive samples were obtained from either a primary or secondary species on the priority list developed as part of the Pacific Flyway Plan.

The identification of H5 and H7 subtypes of avian influenza viruses in wild waterfowl such as those targeted in this surveillance

Table 4. Low-pathogenicity avian influenza H7N3 isolates identified by H7 rRT-PCR testing (n = 359).[A]

Common name	Scientific name	Sex	Age[B]	State	County	Date collected	V.I. subtype[C]
Green-winged teal	*A. crecca*	M	AHY	ID	Bingham	Nov. 4, 2006	H7N3
		F	AHY	ID	Bingham	Dec. 7, 2006	H7N3
		M	U	CA	Fresno	Jan. 3, 2007	H7N3
Mallard	*A. platyrhynchos*	M	HY	NV	Churchill	Dec. 23, 2006	H7N3
Northern pintail	*A. acuta*	F	HY	NV	Churchill	Dec. 29, 2006	H7N3
		M	AHY	NV	Churchill	Jan. 6, 2007	H7N3
Northern shoveler	*A. clypeata*	F	AHY	WA	Clark	Nov. 7, 2006	H7N3
		F	HY	WA	Clark	Nov. 7, 2006	H7N3
		M	HY	WA	Clark	Nov. 7, 2006	H7N3
		M	AHY	WA	Clark	Dec. 7, 2006	H7N3
		M	HY	WA	Clark	Dec. 7, 2006	H7N3
		F	AHY	WA	Clark	Dec. 14, 2006	H7N3
		M	HY	NV	Churchill	Dec. 29, 2006	H7N3
		U	U	CA	Imperial	Jan. 6, 2007	H7N3

[A]Spackman *et al.* (39).
[B]AHY = after hatch year, HY = hatch year, U = unknown.
[C]V.I. subtype = virus isolate subtype.

program is not unexpected. Of the avian influenza viruses isolated in Minnesota from wild birds between 1998 and 2000, 7.4% were H5 (18). Similarly, 7.5% of avian influenza viruses isolated in Northern Europe were H5 subtype (29). Comparison of results between studies needs to be made with care since sample size, age distribution, species, sex, timing and location of collection, and analytic technique may affect the observed avian influenza prevalence rates. For example, within the Pacific Flyway, as many as 25% (161 of 640) of ducks tested in British Columbia were positive for H5 in 2005 (33), but only 15% (219 of 1426) of birds were positive in 2006 (7). Low-pathogenicity H5 viruses including H5N1 have been found on a regular basis in wild birds (19,41). In Europe, H7 avian influenza viruses in wild birds made up 11.1% of viruses isolated (29) but constituted only 1% of the viruses isolated in North America (23).

Most HPAI viruses are thought to arise by successive mutations after LPAI viruses are introduced into poultry, and increased surveillance efforts of domestic and wild birds have identified precursors of HPAI viruses (3). The outbreak of HPAI H7N1 in Italy between 1999 and 2000 was preceded by earlier infection of the flocks with a low-pathogenicity H7N1 virus (8). A closely related LPAI precursor could be found for each of the HPAI outbreaks since 1997 in Europe (30), and similarly in three HPAI outbreaks in the Western Hemisphere: Chile (H7N3), the United States (H5N2), and Canada (H7N3) (37).

The ability to detect HPAI H5N1 in a surveillance effort such as the one we employed was recently demonstrated by Dalessi *et al.* (11), who reported that sampling of live and hunter-killed birds and testing of birds found dead resulted in detection of Switzerland's first known cases of HPAI H5N1 in early 2006. Also in early 2006, Croatian officials reported HPAI H5N1 virus isolated from cloacal swabs of 30 live black-headed gulls (*Larus ridibundus*) that had been sampled as a part of routine surveillance of wild birds on the Adriatic coast (12). These reports follow other detections of HPAI H5N1 in apparently healthy wild birds (9,22) as well as in wild birds found sick and dead (10,17,26).

Experimentally, species of wild waterfowl have been infected with varying results, from mortality in infected individuals to little or no

Table 5. Avian influenza viral subtypes identified from individual H5 rRT-PCR negative samples submitted as part of pooled H5-positive samples to the USDA National Veterinary Services Laboratory for follow up testing (n = 250). All isolates were low-pathogenicity avian influenza.

Common name	Scientific name	Sex	Age[A]	State	County	Date collected	V.I. subtype[B]	No. positive
Mallard	*Anas platyrhynchos*	F	AHY	OR	Lake	Oct. 7, 2006	H5N2	1
		F	AHY	WA	Skagit	Aug. 29, 2006	H4N6	1
		F	HY	MT	Cascade	Sep. 20, 2006	H4N6	1
		F	HY	MT	Cascade	Sep. 19, 2006	H5N3	1
		F	HY	NV	Churchill	Jul. 28, 2006	H3N8	1
		F	HY	NV	Lyon	Aug. 24, 2006	H4N6	1
		F	HY	WA	Skagit	Sep. 8, 2006	H5N2	1
		F	HY	WA	Yakima	Aug. 4, 2006	H4N6	1
		M	HY	MT	Cascade	Sep. 20, 2006	H4N6	1
		M	HY	MT	Cascade	Sep. 20, 2006	H5N3	1
		M	HY	NV	Churchill	Jul. 28, 2006	H3N6	1
		M	HY	NV	Churchill	Jul. 28, 2006	H3N8	4
		M	HY	NV	Lyon	Aug. 24, 2006	H4N6	3
		M	HY	OR	Lake	Sep. 24, 2006	H4N6	1
		M	HY	OR	Multnomah	Aug. 29, 2006	H4N6	1
		M	HY	WA	Skagit	Sep. 8, 2006	H3N8	1
		M	HY	WA	Yakima	Aug. 4, 2006	H10N7	1
Northern pintail	*A. acuta*	M	AHY	UT	Davis	Oct. 7, 2006	H5N2	1

[A]AHY = after hatch year, HY = hatch year.
[B]V.I. subtype = virus isolate subtype.

clinical signs of disease but high shedding of virus (5,20). These results have led to speculation that some species may be capable of transporting HPAI H5N1 over long distances (20). While we did not detect HPAI H5N1 in our study, these recent reports, both from surveillance activities and experimental studies, suggest that wild birds may be important local carriers and may play a role in the local or regional spread of HPAI H5N1 and consequently surveillance in migratory birds is warranted and is a necessary component of any surveillance program.

REFERENCES

1. Alexander, D. J. A review of avian influenza in different bird species. Vet. Microbiol. 74:3–13. 2000.

2. Alexander, D. J. Avian influenza, In: Manual of diagnostic tests and vaccines for terrestrial animals [Internet]. World Organization of Animal Health, Paris. 2005 Available from: http://www.oie.int/Eng/Normes/Mmanual/2008/pdf/2.03.04_AI.pdf. [modified 2005; cited 2008 October 23].

3. Alexander, D. J. An overview of the epidemiology of avian influenza. Vaccine 25:5637–5644. 2007.

4. Becker, W. B. The isolation and classification of Tern virus: Influenza virus A/Tern/South Africa/1961. J. Hyg. 64:309–320. 1966.

5. Brown, J. D., D. E. Stallknecht, J. R. Beck, D. L. Suarez, and D. E. Swayne. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerg. Infect. Dis. 12:1663–1670. 2006.

6. Bub, H. Bird trapping and bird banding: a handbook for trapping methods all over the world. Cornell University Press, Ithaca, New York. 1991.

7. Canadian Cooperative Wildlife Health Center, 2006 Wild Bird Survey [Internet]. Available from: http://www.ccwhc.ca/en/aiv/duck_survey_region_2006.php. [modified 2006; cited 2008 July 22].

8. Capua, I., S. Manangon, M. dalla Pozza, C. Terregino, and G. Cattoli. Avian influenza in Italy 1997–2001. Avian Dis. 47:839–843. 2003.

9. Chen, H., G. J. D. Smith, K. S. Li, J. Wang, X. H. Fan, J. M. Rayner, D. Vijaykrishna, J. X. Zhang, L. J. Zhang, C. T. Guo, C. L. Cheung, K. M. Xu, L. Duan, K. Huang, K. Qin, Y. H. C. Leung, W. L. Wu, H. R. Lu, Y. Chen, N. S. Xia, T. S. P. Naipospos, K. Y. Yuen, S. S. Hassan, S. Bahri, T. D. Nguyen, R. G. Webster, J. S. M. Peiris, and Y. Guan. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc. Natl. Acad. Sci. U.S.A. 103:2845–2850. 2006.

10. Chen, H., G. J. D. Smith, S. Y. Zhang, K. Qin, J. Wang, K. S. Li, R. G. Webster, J. S. M. Peiris, and Y. Guan. H5N1 virus outbreak in migratory waterfowl. Nature 436:191–192. 2005.

11. Dalessi, S., R. Hoop, and M. Engels. The 2005/2006 avian influenza monitoring of wild birds and commercial poultry in Switzerland. Avian Dis. 51:355–358. 2007.

12. Department for Environment, Food and Rural Affairs (DEFRA). Situation analysis—outbreaks of HPAI H5N1 virus in Europe during 2005/2006—an overview and commentary. 2006 [modified 2006; cited 2009 February 2]. Available from: http://collections.europarchive.org/tna/2008017205404/http://defra.gov.uk/animalh/diseases/monitoring/pdf/hpai-europe300606.pdf

13. Docherty, D. E., and P. G. Slota. 1988. Use of muscovy duck embryo fibroblasts for the isolation of viruses from wild birds. J. Tiss. Cult. Meth. 11:165–170. 1988.

14. Ellis, T. M., R. B. Bousfield, L. A. Bissett, K. C. Dyrting, G. S. M. Luk, S. T. Tsim, K. Sturm-Ramirez, R. G. Webster, Y. Guan, and J. S. M. Peiris. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol. 33:492–505. 2004.

15. Feare, C. J. The spread of avian influenza. Ibis 149:424–425. 2007.

16. Fergus, R., M. Fry, W. B. Karesh, P. P. Marra, S. Newman, and E. Paul. Migratory birds and avian flu. Science 312:845. 2006.

17. Goujgoulova, G., and N. Oreshkova. Surveillance on avian influenza in Bulgaria. Avian Dis. 51:382–386. 2007.

18. Hanson, B. A., D. E. Stallknecht, D. E. Swayne, L. A. Lewis, and D. A. Senne. Avian influenza viruses in Minnesota ducks during 1998–2000. Avian Dis. 47:867–871. 2003.

19. Jonassen, C. M., and K. Handeland. Avian influenza virus screening in wild waterfowl in Norway, 2005. Avian Dis. 51:425–428. 2007.

20. Keawcharoen, J., D. van Riel, G. van Amerongen, T. Bestebroer, W. E. Beyer, R. van Lavieren, A. D. M. E. Osterhaus, R. A. M. Fouchier, and T. Kuiken. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). Emerg. Infect. Dis. 14:600–607. 2008.

21. Koehler, A. V., J. M. Pearce, P. L. Flint, J. C. Franson, and H. S. Ip. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). Mol. Ecol. 17:4754–4762. 2008.

22. Kou, Z., F. M. Lei, J. Yu, Z. J. Fan, Z. H. Yin, C. X. Jia, K. J. Xiong, Y. H. Sun, X. W. Zhang, X. M. Wu, X. B. Gao, and T. X. Li. New genotype of avian influenza H5N1 viruses isolated from tree sparrows in China. J. Virol. 79:15460–15466. 2005.

23. Krauss, S., D. Walker, S. P. Pryor, L. Niles, L. Chenghong, V. S. Hinshaw, and R. G. Webster. Influenza A viruses of migrating wild aquatic birds in North America. Vector Borne Zoonotic Dis. 4:177–189. 2004.

24. Kwon, Y. K., S. J. Joh, M. C. Kim, Y. J. Lee, J. G. Choi, E. K. Lee, S. H. Wee, H. W. Sung, J. H. Kwon, M. I. Kang, and J. H. Kim. Highly pathogenic avian influenza in magpies (*Pica pica sericea*) in South Korea. J. Wildl. Dis. 41:618–623. 2005.

25. Lee, Y.-J., Y.-K. Choi, Y.-J. Kim, M.-S. Song, O.-M. Jeong, E.-K. Lee, W.-J. Jeon, W. Jeong, S.-J. Joh, K.-S. Choi, M. Her, M.-C. Kim, A. Kim, M.-J. Kim, E. H. Lee, T.-G. Oh, H.-J. Moon, D.-W. Yoo, J.-H. Kim, M.-H. Sung, H. Poo, J.-H. Kwon, and C.-J. Kim. Highly pathogenic avian influenza virus (H5N1) in domestic poultry and relationship with migratory birds, South Korea. Emerg. Infect. Dis. 14:487–490. 2008.

26. Lei, F., S. Tang, D. Zhao, X. Zhang, Z. Kou, Y. Li, Z. Zhang, Z. Yin, S. Chen, S. Li, D. Zhang, B. Yan, and T. Li. Characterization of H5N1 influenza viruses isolated from migratory birds in Qinghai Province of China in 2006. Avian Dis. 51:568–572. 2007.

27. Liu, J., J. H. Xiao, F. Lei, Q. Zhu, K. Qin, X.-W. Zhang, X.-L. Zhang, D. Zhao, G. Wang, Y. Feng, J. Ma, W. Liu, J. Wang, and G. F. Gao. Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 309:1206. 2005.

28. Makarova, N. V., N. V. Kaverin, S. Krauss, D. Senne, and R. G. Webster. Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. J. Gen. Virol. 80:3167–3171. 1999.

29. Munster, V. J., C. Baas, P. Lexmond, J. Waldenstöm, A. Wallensten, T. Fransson, G. F. Rimmelzwaan, W. E. P. Beyer, M. Schutten, B. Olsen, A. D. M. E. Osterhaus, and R. A. M. Fouchier. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Pathogen 3:e61. 2007.

30. Munster, V. J., A. Wallensten, C. Baas, G. F. Rimmelzwaan, M. Schutten, B. Olsen, A. D. M. E. Osterhaus, and R. A. M. Fouchier. Mallards and highly pathogenic avian influenza ancestral viruses, Northern Europe. Emerg. Infect. Dis. 11:1545–1551. 2005.

31. Normile, D. Evidence points to migratory birds in H5N1 spread. Science 311:1225. 2006.

32. Pacific Flyway Council. Surveillance for early detection of highly pathogenic avian influenza H5N1 in wild migratory birds: a strategy for the Pacific Flyway [Internet]. Available from: http://www.pacificflyway.gov/Documents/AIS_plan.pdf. [modified 2006; cited 2008 July 22].

33. Parmley, E. J., N. Bastien, T. F. Booth, V. Bowes, P. A. Buck, A. Breault, D. Caswell, P.-Y. Daoust, J. C. Davies, S. M. Elahi, M. Fortin, F. Kibenge, R. King, Y. Li, N. North, D. Ojkic, J. Pasick, S. P. Pryor, J. Robinson, J. Rodrigue, H. Whitney, P. Zimmer, and F. A. Leighton. Wild bird influenza survey, Canada, 2005. Emerg. Infect. Dis. 14:84–87. 2008.

34. Saad, N. D., L. S. Ahmed, M. A. Gamal-Eldein, M. K. Fouda, F. M. Khalil, S. L. Yingst, M. A. Parker, and M. R. Montevillel. Possible avian influenza (H5N1) from migratory bird, Egypt. Emerg. Infect. Dis. 13:1120–1121. 2007.

35. Salzberg, S. L., C. Kingsford, G. Cattoli, D. J. Spiro, D. A. Janies, M. M. Aly, I. H. Brown, E. Couacy-Hymann, G. M. De Mia, D. H. Dung, A. Guercio, T. Joannis, A. S. M. Ali, A. Osmani, I. Padalino, M. D. Saad, V. Savic, N. A. Sengamalay, S. Yingst, J. Zaborsky, O. Zorman-Rojs, E.

Ghedin, and I. Capua. Genome analysis linking recent European and African influenza (H5N1) viruses. Emerg. Infect. Dis. 13:713–718. 2007.

36. Senne, D. A., B. Panigraphy, Y. Kawaoka, J. E. Pearson, J. Suss, M. Lipkind, H. Kida, and R. G. Webster. Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. Avian Dis. 40:425–437. 1996.

37. Senne, D. A., D. L. Suarez, D. E. Stallknecht, J. C. Pedersen, and B. Panigrahy. Ecology and epidemiology of avian influenza in North and South America. Dev. Biol. (Basel) 124:37–44. 2006.

38. Spackman, E., H. S. Ip, D. L. Suarez, R. D. Slemons, and D. E. Stallknecht. Analytical validation of a real-time reverse transcription polymerase chain reaction test for Pan-American lineage H7 subtype avian influenza viruses. J. Vet. Diagn. Invest. 20:612–616. 2008.

39. Spackman, E., K. G. McCracken, K. Winker, and D. E. Swayne. An avian influenza virus from waterfowl in South America contains genes from North American avian and equine lineages. Avian Dis. (Suppl.) 51:273–274. 2007.

40. Spackman, E., D. E. Swayne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L. Perdue, K. Lohman, L. T. Daum, and D. L. Suarez. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. 40:3256–3260. 2002.

41. Spackman, E., D. E. Swayne, D. L. Suarez, D. A. Senne, J. C. Pederson, M. L. Killian, J. Pasick, K. Handel, S. P. Somanathan Pillai, C.-W. Lee, D. Stallknecht, R. Slemons, H. S. Ip, and T. Deliberto. Characterization of low-pathogenicity H5N1 avian influenza viruses from North America. J. Virol. 81:11612–11619. 2007.

42. Starick, E., M. Beer, B. Hoffmann, C. Staubach, O. Werner, A. Globig, G. Strebelow, C. Grund, M. Durban, F. J. Conraths, T. Mettenleiter, and T. Harder. Phylogenetic analyses of highly pathogenic avian influenza virus isolates from Germany in 2006 and 2007 suggest at least three separate introductions of H5N1 virus. Vet. Microbiol. 128:243–252. 2008.

43. U.S, Interagency Working Group. An early detection system for highly pathogenic H5N1 avian influenza in wild migratory birds: U.S. Interagency Strategic Plan [Internet]. Washington, DC. 2006. Available from: http://www.doi.gov/issues/birdflu_strategicplan.pdf [modified 2006; cited 2008 November 05].

44. Wallensten, A., V. J. Munster, N. Latorre-Margalef, M. Brytting, J. Elmberg, R. A. M. Fouchier, T. Fransson, P. D. Haemig, M. Karlsson, A. Lundkvist, A. D. M. E. Osterhaus, M. Stervander, J. Waldenström, and B. Olsen. Surveillance of influenza A virus in migratory waterfowl in Northern Europe. Emerg. Infect. Dis. 13:404–411. 2007.

45. Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. Evolution and ecology of influenza A viruses. Microbiol. Rev. 56:152–179. 1992.

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